Cytotoxicity of Methylsulfonylmethane (MSM) on Gastric Cancer (AGS) and Liver Cancer (HepG2) Cell Lines

Department of Pharmacology, School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran

Naser Jafari[1], Shahab Bohlooli[2] , Reza Rostami[3], Negar Molazadeh[4]
1. Biochemistry Student (M.Sc.) 2. Ph.D of Pharmacology, Assistant Professor
3.Nursing Student,4.Medicine Student

ESMO CONFERENCE
11TH WORLD CONGRESS on Gastrointestinal CANCER
Ardabil University of Medical Sciences

Background:

Dimethyl sulfone (DMSO2) also known as Methylsulfonyl-methane (MSM) and Methyl sulfone is an organic sulfur containing compound that occurs naturally in a variety of fruits and vegetables. MSM has been proven to have anti-inflammatory and anti oxidant properties in an in vitro study. [1] Two other studies demonstrated a significant reduction in the time to tumor onset in rats treated with MSM. [2-3] It has been suggested that MSM has a chemopreventive mechanism that effects the interaction of tumor cells with the host immune response. [4] More recently an in vitro study showed both MSM and aspirin induce terminal differentiation, utilizing cyclooxigenase independent mechanism. [5] Although aspirin was used at a low dose in this study, MSM was used in a much higher concentration to induce a higher level of differentiation, thereby dismantling the cellular capacity for proliferation. MSM reduces the binding, uptake and degradation of low density lipoproteins by cultured fibroblasts. [6] Another study showed dimethyl suloxide (DMSO) and MSM caused dose dependent suppression of growth and proliferative properties of aortic smooth muscle and endothelial cells in vitro, [7] in addition MSM effects were more potent and irreversible than the effects of DMSO. Although not well studied, MSM has been used clinically to treat conditions such as snoring, scleroderma, fibromyalgia, systemic lupus erythematosus, repetitive stress injuries and osteoarthritis. [8] MSM is believed to be non toxic. [9] A 30 day study that used a 2600 mg per day dosage revealed no side effects. [10] However no study has examined long term supplementation with MSM. One study of MSM in rats showed that oral administration at a dose of 1.5 g/kg/day for 90 days did not cause any adverse side effects or increased mortality. [11] Considering chemical properties, the cytotoxic effects or anti cancer activity of MSM has not been investigated on human cancer cells in vitro, and it is not used for the management or treatment of cancer.Gastric cancer is the fourth most common cancer and second leading cause of cancer death worldwide. [12] The gastric adenocarcinoma is the most common subtype of gastric cancer. [13] Although gastric cancer has been considered as a chemosensitive tumor for many years, no significant progress in its management has resulted within the last two decades. [14] The hepatocellular carcinoma is considered to be one of the most common malignant tumors and the therapeutic effectiveness of drugs in clinical use at present is rather low. [15]

Considering chemosensitive properties of gastric and liver cancers, and chemopreventive characteristics of MSM, we investigated in vitro cytotoxic effects of MSM on these cancer cell lines.

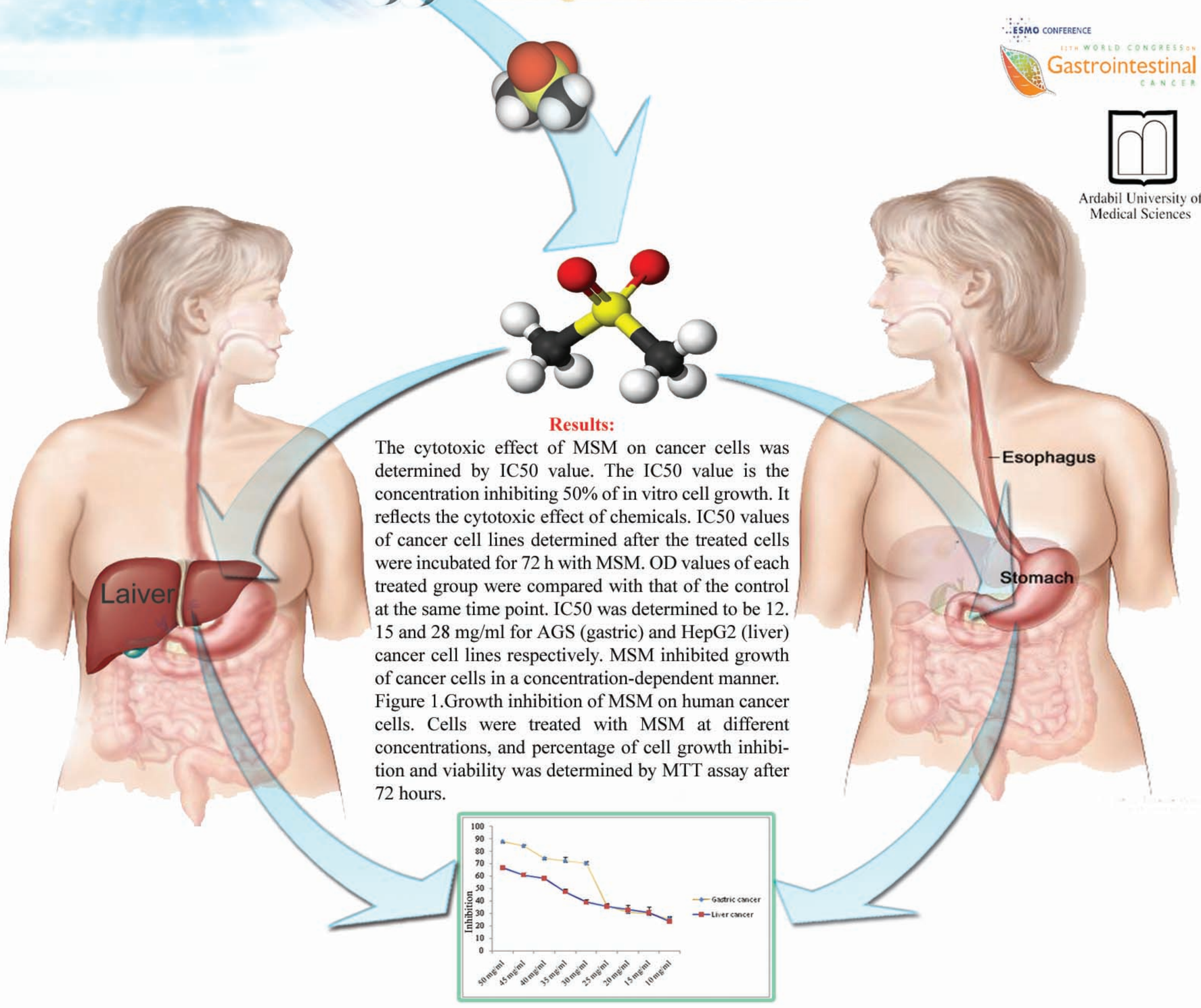
Methods:

The human cancer cell lines (AGS and HepG2) were provided from Pasteur Institute of Iran Cell Bank (Tehran, Iran). All reagents and medium were prepared just before use.

Cell culture: The cells were cultured in RPMI-1640 (Cat. No: 51800-035, GIBCO, UK) medium supplemented with 10% FBS (Cat. No: 10270-106, GIBCO, UK), penicillin 100 unit/ml and streptomycin 100 µg/ml. The cells were incubated at 37 C in a water saturated atmosphere of 5% CO2 and 95% air until confluence. After one weak the cells were removed with a solution containing 0.25 (w/v) trypsin and 0.02 (w/v) EDTA. After detaching, the cell suspensions plated in 96-well plates at a density of $1x10^4$ cells per well in 200 µl medium. Plates were incubated for an overnight, then the medium was removed and cells were treated with a medium (without FBS) containing 50 mg/ml MSM (Cat. No: 41631, Fluka, UK) by ¼ serial dilutions, then plates were incubated for 72 h.

Cytotoxicity assay (MTT assay): The cytotoxicity of MSM was determined by MTT (Merck, Germany) assay. After 3 days, supernatant medium was removed, then 20 µl of 5 mg/ml MTT and 180 µl medium were added to each well, plates incubated again for 4 h to completing 72 h incubation time. The supernatants were removed and 200 µl DMSO was added to each well. Plates were then shaken for 10 min. The absorbance at 540 nm was measured by microplate reader (Synergy HT, BioTek), using wells without cells as blank.

Statistical analysis: IC50 values were obtained using Sigma Plot 11 software. Mean data values for the growth inhibition study are expressed as the mean ± S.E. from the mean.



Results:

The cytotoxic effect of MSM on cancer cells was determined by IC50 value. The IC50 value is the concentration inhibiting 50% of in vitro cell growth. It reflects the cytotoxic effect of chemicals. IC50 values of cancer cell lines determined after the treated cells were incubated for 72 h with MSM. OD values of each treated group were compared with that of the control at the same time point. IC50 was determined to be 12. 15 and 28 mg/ml for AGS (gastric) and HepG2 (liver) cancer cell lines respectively. MSM inhibited growth of cancer cells in a concentration-dependent manner.

Figure 1.Growth inhibition of MSM on human cancer cells. Cells were treated with MSM at different concentrations, and percentage of cell growth inhibition and viability was determined by MTT assay after 72 hours.

Discussion:

In the present study, we demonstrated that MSM behave in a cytotoxic manner towards the cancer cell lines in vitro and act as potent apoptosis inducing agent. MSM had cytotoxic effects against gastric (AGS) and liver (HepG2) cancer cell lines. MSM exerted its greatest toxic effect on the gastric (AGS) cell line, killing 50% of AGS cells after 72 hours exposure. The results demonstrated that MSM has a strong anticancer and cytotoxic effect by inducing apoptotic cell death. MSM might induces apoptotic signaling pathway by activation of caspases, however further studies should be conducted to elucidate the precise mechanism of MSM-induced apoptosis in cancer cells especially in gastric cancer.

The anticancer activity of MSM against human cancer cells might result, at least in part, from inhibition of DNA synthesis and proliferation, and from activation and/or suppression of gene expression. In conclusion, the present study demonstrated that MSM exhibited cytotoxic effect on cultured human cancer cells because of the induction of apoptosis. This finding provide a new understanding of the cytotoxic effects on gastric and liver cancer cells caused by MSM.

MSM needs to be investigated further, especially with animal tumor models to confirm its anticancer and chemotherapeutic activity in vivo.

References:

[1] Beilke MA, Collins-Lech C, Sohnle PG. (1987) Effects of dimethyl sulfoxide on the oxidative function of human neutrophils J Lab Clin Med. 110: 91-96.
[2] O'Dwyer PJ, McCabe DP, Sickle-Santanello B J, et al. (1988) Use of polar solvents in chemoprevention of 1,2-dimethylhydrazine induced colon cancer. Cancer. 62: 944-948.
[3] McCabe D, O'Dwyer P, Sickle-Santanello B, et al. (1986) Polar solvents in the chemoprevention of Dimethylbenzanthracene induced rat mammary cancer. Arch Surg. 121: 1455-1459.
[4] Cox WI, Specter S, Friedman H. (1982) Susceptibility of Friend erythroleukemia cells to natural cytotoxicity after in vitro treatment with dimethyl sulfoxide. Proc Soc Exp Biol Med. 169: 337-342.
[5] Ebisuzaki K. (2003) Aspirin and methylsulfonyl-methane (MSM): a search for common mechanisms, with implications for cancer prevention. Anticancer Res. 23: 453-458.
[6] Alam SS, Layman DL. (1982) Dimethyl sulfoxide as a cholesterol-lowering agent in cultured fibroblasts exposed to low density lipoproteins in culture. Biochim Biophys Acta. 710:306-313.
[7] Layman DL. (1987) Growth inhibitory effects of dimethyl sulfoxide and dimethyl sulfone on vascular smooth muscle and endothelial cells in vitro. In Vitro Cell Dev Biol. 23: 422-428.
[8] Jacob SW, Appleton J. (2003) MSM: The Definitive Guide. A Comprehensive Review of the Science and Therapeutics of Methylsulfonylmethane. Topanga, CA: Freedom Press:107-121.
[9] Morton JI, Siegel BV. (1986) Effects of oral dimethyl sulfoxide and dimethyl sulfone on murine autoimmune lymphoproliferative disease. Proc Soc Exp Biol Med. 183: 227-230.
[10] Barrager E, Veltmann JR Jr, Schauss AG, Schiller RN. (2002) A multicentered, open-label trial on the safety and efficacy of methylsulfonylmethane in the treatment of seasonal allergic rhinitis. J Altern Complement Med. 8:167-173.
[11] Horvath K, Noker PE, Somfai-Relle S, et al. (2002) Toxicity of methylsulfonylmethane in rats. Food Chem Toxicol. 40:1459-1462.
[12] Crew KD, Neugut AI. (2006) Epidemiology of gastric cancer. World J Gastroenterol 12:354-362.
[13] Alberts SR, Cervantes A, van de Velde CJ. (2003) Gastric cancer: epidemiology, pathology and treatment. Ann Oncol 14 Suppl 2:ii31-36.
[14] Cervantes A, Rosello S, Roda D, Rodriguez-Braun E. (2008) The treatment of advanced gastric cancer: current strategies and future perspectives. Ann Oncol 19 Suppl 5:v103-107
[15] Graziadei, I., Kelly, T., Schirmer, M., Geisen, F.H., Vogel, W., Konwalink, G., (1998). Antitumor effect of the nucleoside analogs 2-chlorodeoxyadenosine and 2,2_difluorodeoxycytidine on human hepatoma Hep G2 cells. Journal of Hepatology 28, 504–509.

Designed by: M.Sarvgamat